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Effects of pre-sowing treatments and altitudes on seedling emergence *of Zanthoxylum oxyphyllum* Edgew

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Abstract
The purpose of this study was to investigate the effects of different treatments on the germination rate and shorten the germination period of *Zanthoxylum Oxyphyllum* Edgew. The experiment was performed in two different altitudes (857 m and 1000 m A.S.L) and 7 different pretreatments *viz*., sulfuric acid scarification (98%) for 10 min, mechanical scarification, seed priming with gibberellic acid (2000ppm) for 24 hours, cold stratification for 1 month at 3 ºC, hot water treatment (80 ºC) for 10 min, hot ash treatment (135 ºC) for 5 min and control. Our findings indicate that the overall average percentage was higher at 857 metres above mean sea level compared to 1500 metres above sea level. Seed treatment with gibberellic acid (2000ppm) was the most effective pretreatment for both altitudes followed by cold stratification with germination percentage of 45.95% and 18.13% respectively.

Keywords: Seed, germination period, altitudes, pretreatments

Introduction

Botanically classified as *Zanthoxylum oxyphyllum*, grows as a deciduous tree that can reach up to 15-20 meters in height and belongs to the Rutaceae, or citrus family. Commonly known as Mezenga (Assamese)/Onger (Mishing), Indian Ivy-rue, Ma Kwaen, Prickly Ash and the Indian pepper, there are over two hundred species of Zanthoxylums, many of which bear a fruit that is used to make Szechwan pepper (Singh *et al*, 2000) [10]. The different parts of the plants: leaves, fruits, stem, bark, seeds have been used in several indigenous medicinal practices as carminative, antipyretic, appetizer, stomachic, toothache, dyspepsia. Tender shoots and leaves of this plant are taken as vegetable and in non-vegetarian dishes. They have been found to aid against stomach disorder, act as a blood purifier as well as against leucoderma (Kusari *et al*., 2013) [7]. Fruits of *Z. oxyphyllum* are used as spice and are known to help in digestive disorders. The bark of the plant is commonly applied to treat skin diseases, rheumatism, ulcers, varicose veins, leg aches, inflammations, fever, and hypotension. In addition to this, it also has stimulant, astringent, and digestive properties and is used for the treatment of dyspepsia and diarrhoea (Konwar and Bordoloi, 2011) [2].

Species of *Zanthoxylum* are of great economic importance as source of edible fruit, oil, wood, raw materials for industries, medicinal plant, ornamental, culinary application. This diverse characteristic results in huge demand of *Zanthoxylum* in both domestic and international market due to which the market price has been escalating in the last two decades (Venieraki, 2017) [12]. Despite the species importance, a comprehensive review on *Zanthoxylum* is still not available and one of the major hinderance lies in its long germination period. It is generally propagated through seeds, and from vegetative parts through soft wood cuttings. Natural regeneration usually occurs through seeds i.e., freshly harvested seeds are best for large-scale cultivation, but the seeds undergo strong dormancy and may take few months to years for germination (Dirr *et al*., 1987) [3]. Due to the long germination period efforts were made to shorten the length of germination and to increase the germination rate by experimenting it with 7 different pretreatments, 4 soil medium and 2 different altitudes.

Materials and Methods

Matured fresh seeds of *Z. oxyphyllum* were collected from Penkim village, Kiphire district which is situated at 25.7999^0 N Latitude and 94.9113^0 E longitude at an elevation of 2157-meters above sea level. Research work was carried out at two different altitudes i.e Kandinu station, Tseminyu at elevation of 857 meters and Pfutseromi, Phek at an elevation of 1500 meters above the mean sea level.

The total number of seeds (2800 seeds) were soaked in water for 24 hours, all of which passed the floating test, and were divided into 7 different treatments as follows:

Treatment 1: Sulfuric Acid scarification (400 Seeds)
The seeds were gently stirred in high conc. 98% for a short period (approx. 3 minutes). After which the seeds were taken out, washed in running water and sown. The treated seeds were thoroughly rinsed four times with sterile water before sowing.

Treatment 2: Mechanical scarification (400 Seeds)
The testa was physically opened by nicking it with a knife to allow moisture and air in.

Treatment 3: Seed priming with Gibberellic Acid (400 seeds)
The seeds were treated 2000 ppm (stock solution of 10 mg of GA in 10 ml of water) for 24 hours. The treated seeds were thoroughly rinsed four times with sterile water before sowing as mentioned before.

Treatment 4: Cold Stratification (400 seeds)
Cold stratification treatment was performed by placing seeds in air-tight plastic bags containing damp sand as a medium and kept in the refrigerator at a temperature of 4 ºC for two months.

Treatment 5: Hot water treatment (400 seeds)
The seeds were submerged into the hot water (80 degree Celsius) for 10 minutes. The seeds were kept submerged till the water reached room temperature.

Treatment 6: Hot ash treatment (400 seeds): The seeds were introduced in 100-degree Celsius hot ash for 5 minutes.

Treatment 7: Control (400 seeds)
Seeds were sowed immediately after the 24 hours soaking time.

Data was collected daily 15 days after sowing (DAS) i.e., May 19th 2023 to 6th July 2023. Throughout the study period, the data obtained was analyzed using the germination percentage formula,

$$\text{Germination percentage: } \frac{\text{Seeds germinated}}{\text{Total seeds}} \times 100$$

Results

Treatment results from Pfutseromi, Phek (1500 m A.S.L)
There was a significant effect from all pretreatments on the germination and seedling emergence of *Z. oxyphyllum* Edgew (Table.1). The results indicated that total germination percentage of Gibberellic acid (T_3) was the highest among all the other treatments while traditional hot ash treatment (T_6) and control (T_7) reported the lowest germination. All treatments were superior than control in regard with the length of germination. Germination in control was observed only after 40 DAS. The highest germination percentage was recorded with gibberellic acid (T_3) in topsoil + fym soil medium with an overall percentage of 45.95% closely followed by gibberellic acid in topsoil with 39.13%. All pre-sowing treatment with gibberellic acid indicated superior germination percent i.e., gibberellic acid (T_3) in subsoil + fym (20.75%) and gibberellic acid (T_3) in subsoil (13.28%) while other treatments such as cold stratification (T_4) with topsoil recorded germination percentage of 14.05%, hot water treatment (T_5) in topsoil+ fym recorded 8.05% and treatment such as Sulfuric acid (T_1) and control (T_7) recorded less than 5% germination. No germination was recorded during the first week of sowing.

Table 1: Germination percentage of *Z. oxyphyllum* Edgew at Pfutseromi, Phek (1500 m A.S.L)

Treatment	Soil type	25 DAS	35 DAS	45 DAS	55 DAS	65 DAS	Mean Avg.	Std. Deviation	Total percentage
Sulfuric acid (T_1)	Top soil	0	0.00	0.85	1.73	3.25	1.17	0.34	5.83
Sulfuric acid (T_1)	Sub soil	0	0.00	0.00	0.43	1.78	0.44	0.24	2.20
Sulfuric acid (T_1)	Top soil+ FYM	0	0.00	0.10	0.90	2.50	0.70	0.23	3.50
Sulfuric acid (T_1)	Sub soil+FYM	0	0.00	0.00	0.00	0.85	0.17	0.20	0.85
Mechanical scarification (T_2)	Top soil	0	0.13	1.25	2.20	2.30	1.18	0.26	5.88
Mechanical scarification (T_2)	Sub soil	0	0.00	0.00	0.05	0.25	0.06	0.05	0.30
Mechanical scarification (T_2)	Top soil+ FYM	0	0.35	1.08	1.70	2.10	1.05	0.12	5.23
Mechanical scarification (T_2)	Sub soil+FYM	0	0.05	1.28	1.80	2.28	1.08	0.10	5.40
Gibberellic acid (T_3)	Top soil	0	3.93	6.93	12.05	16.23	7.83	0.70	39.13
Gibberellic acid (T_3)	Sub soil	0	1.05	2.60	4.03	5.55	2.65	0.13	13.23
Gibberellic acid (T_3)	Top soil+ FYM	0	4.80	10.08	14.00	17.08	9.19	0.72	45.95
Gibberellic acid (T_3)	Sub soil+FYM	0	0.43	3.58	7.70	9.05	4.15	0.62	20.75
Cold stratification (T_4)	Top soil	0	1.48	2.83	4.33	5.43	2.81	0.31	14.05
Cold stratification (T_4)	Sub soil	0	0.00	0.00	0.25	0.75	0.20	0.11	1.00
Cold stratification (T_4)	Top soil+ FYM	0	1.35	2.38	3.60	4.00	2.27	0.26	11.33
Cold stratification (T_4)	Sub soil+FYM	0	0.75	2.25	3.10	3.25	1.87	0.30	9.35
HWT (T_5)	Top soil	0	0.00	0.00	0.45	2.15	0.52	0.37	2.60
HWT (T_5)	Sub soil	0	0.00	0.00	0.00	0.20	0.04	0.05	0.20
HWT (T_5)	Top soil+ FYM	0	0.30	1.45	2.50	3.80	1.61	0.17	8.05
HWT (T_5)	Sub soil+FYM	0	0.00	0.00	0.33	1.35	0.34	0.20	1.68
Traditional hot ash treatment (T_6)	Top soil	0	0.00	0.00	0.00	0.43	0.09	0.16	0.43
Traditional hot ash treatment (T_6)	Sub soil	0	0.00	0.00	0.50	1.43	0.39	0.18	1.93
Traditional hot ash treatment (T_6)	Top soil+ FYM	0	0.00	0.00	0.18	1.55	0.35	0.18	1.73
Traditional hot ash treatment (T_6)	Sub soil+FYM	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Control (T_7)	Top soil	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control (T_7)	Sub soil	0	0.00	0.00	0.00	0.20	0.04	0.10	0.20
Control (T_7)	Top soil+ FYM	0	0.00	0.00	0.00	0.23	0.05	0.16	0.23
Control (T_7)	Sub soil+FYM	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Average percentage									7.18

Treatment results from Kandinu station, Tseminyu (857 M A.S.L.): The experiment in Kandinu station indicated similar results. There was significant effect from Gibberellic acid treatment (T_3) in the germination and seedling emergence of *Z. oxyphyllum* Edgew (Table.2). The highest was seen from Gibberellic acid (T_3) in topsoil + fym soil medium with an overall germination percentage of 42.35% followed by gibberellic acid in sub soil + fym with 27.80%. Treatment with cold stratification (T_4) also proved efficient with the highest in topsoil+fym medium (18.13%) and lowest in subsoil media (3.75%). Other treatments such as mechanical stratification (T_2) with topsoil recorded germination of 12.63% closely followed by sulfuric acid treatment(T_2) and hot water treatment (T_5) in topsoil+ fym with germination percent of 10.83 and 10.20% respectively. In this experiment, control (T_7) resulted in higher germination and seedling emergence as compared to the other experimental site with 1500 m a.s.l. The length of germination was also significantly reduced in all treatments as compared with control. The treatment, T_3 resulted in the shortest length of germination i.e., within 25 DAS, other treatments such as cold stratification, hot water treatment (HWT) and mechanical scarification also resulted in seedling emergence within 25 DAS.

Table 2: Germination percentage of *Z. oxyphyllum* Edgew at Kandinu station, Tseminyu (857 m A.S.L.)

Treatment	Soil type	25 DAS	35 DAS	45 DAS	55 DAS	65 DAS	Mean Avg.	Std. Deviation	Total Percentage
Sulfuric acid (T_1)	Top soil	0.00	0.00	0.00	0.38	1.30	0.34	0.14	1.68
Sulfuric acid (T_1)	Sub soil	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sulfuric acid (T_1)	Top soil+ FYM	0.00	0.13	1.70	3.90	5.10	2.17	0.26	10.83
Sulfuric acid (T_1)	Sub soil+FYM	0.00	0.00	0.38	3.10	3.23	1.34	2.06	6.70
Mechanical scarification (T_2)	Top soil	0.00	0.20	0.25	0.70	1.55	0.54	0.15	2.70
Mechanical scarification (T_2)	Sub soil	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mechanical scarification (T_2)	Top soil+ FYM	0.10	1.58	2.33	3.53	5.10	2.53	0.12	12.63
Mechanical scarification (T_2)	Sub soil+FYM	0.00	0.43	1.50	3.13	4.30	1.87	0.16	9.35
Gibberellic acid (T_3)	Top soil	0.05	1.13	5.18	6.95	8.83	4.43	2.84	22.13
Gibberellic acid (T_3)	Sub soil	0.55	2.03	4.85	7.28	9.58	4.86	0.24	24.28
Gibberellic acid (T_3)	Top soil+ FYM	1.50	6.78	10.08	11.20	12.80	8.47	0.55	42.35
Gibberellic acid (T_3)	Sub soil+FYM	1.08	4.70	5.83	7.08	9.13	5.56	0.39	27.80
Cold stratification (T_4)	Top soil	0.00	0.00	0.38	2.20	3.90	1.30	0.31	6.48
Cold stratification (T_4)	Sub soil	0.00	0.00	0.40	0.90	2.45	0.75	0.30	3.75
Cold stratification (T_4)	Top soil+ FYM	0.23	1.28	3.80	5.98	6.85	3.63	0.49	18.13
Cold stratification (T_4)	Sub soil+FYM	0.08	0.73	2.14	3.85	5.08	2.37	0.37	11.86
HWT (T_5)	Top soil	0.05	0.90	2.05	2.65	3.78	1.89	0.18	9.43
HWT (T_5)	Sub soil	0.05	0.65	1.43	2.25	2.70	1.42	0.08	7.08
HWT (T_5)	Top soil+ FYM	0.30	1.43	2.00	2.90	3.58	2.04	0.08	10.20
HWT (T_5)	Sub soil+FYM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Traditional hot ash treatment (T_6)	Top soil	0.00	0.15	0.90	1.50	2.08	0.93	0.11	4.63
Traditional hot ash treatment (T_6)	Sub soil	0.00	0.03	0.48	1.20	1.55	0.65	0.09	3.25
Traditional hot ash treatment (T_6)	Top soil+ FYM	0.00	0.00	0.63	0.95	1.43	0.60	0.11	3.00
Traditional hot ash treatment (T_6)	Sub soil+FYM	0.00	0.00	0.00	0.10	0.90	0.20	0.14	1.00
Control (T_7)	Top soil	0.00	0.00	0.00	0.00	0.65	0.13	0.14	0.65
Control (T_7)	Sub soil	0.00	0.00	0.00	0.00	0.28	0.06	0.06	0.28
Control (T_7)	Top soil+ FYM	0.00	0.00	0.00	0.00	0.35	0.07	0.11	0.35
Control (T_7)	Sub soil+FYM	0.00	0.00	0.00	0.00	0.55	0.11	0.10	0.55
Average Percentage									8.61

Discussion

According to the results obtained in both altitudes i.e., Pfutseromi, Phek at an elevation of 1500 meters and Kandinu station, Tseminyu at elevation of 857 meters above the mean sea level, gibberellic acid in topsoil+fym medium gave the best results with an overall germinating percentage of 45.95% and 42.35% respectively. Gibberellic acid (GA or GA3) is a natural phytohormone that promotes cell growth and is critical to breaking dormancy and triggering germination. Furthermore, gibberellic acid with all soil medium proved more effective to break the seed dormancy of *Z. oxyphyllum* Edgew (Table 1 and 2). The results clearly suggest for seed germination ecology of *Z. oxyphyllum* to formulate seed pre-treatments protocol for mass multiplication along with short germination period. It also accelerates the regeneration potential of the species to conserve its diversity in the nature. It is a simple, inexpensive, and convenient method for improving and obtaining uniform germination and seedling emergence for raising nursery (Ganga *et al*., 2017) [5].

Treatment of seeds with cold stratification techniques by subjecting them to low temperature (4-6 ºC) for prolonged periods of time (up to 90 days in some species) has proven to be effective on enzymatic activity stimulation and helped promote germination in many tree seeds around the world (Nawrot-Chorabik *et al*., 2021) [8]. The present study also confirms the significance of cold stratification as a dormancy breaking treatment for the germination of *Z. oxyphyllum*. The records obtained during the experiment shows a clear indication of slow but steady increase in germination in all soil types with the highest germination percent of 18.13%. Other treatments such as sulfuric acid treatment, hot water treatment and mechanical stratification also proved superior in germination compared to control. Moreover, pre-treatment before sowing through various techniques can offer a significant contribution towards improved seed germination, uniform seedling growth and enhancing optimum field establishment (Güleryüz *et al*., 2021) [6], this is in line with our experiment where all pre-sowing treatments (i.e., T_1-T_6) resulted in improved seed germination.

The low altitude (i.e., Kandinu station, Tseminyu, 857 m a.s.l.) resulted in higher average germination percent (8.61%) as compared with high altitude (Pfutseromi, Phek, 1500 m a.s.l). This may be due to the hot and humid climatic conditions prevalent in the region. In a similar study, the highest percentage average of seed germination at the rate of 80% for altitude 1700 and the lowest 52.5% for the altitude 2700 meters above sea level (Salehani *et al*., 2013) [9]. Reproductive biology, particularly germination characteristics, is crucial to understanding how species cope with environmental variation (Bauk *et al*. 2017) [1] because it could vary depending on altitude and other factors. Therefore, a comparison of the germination capacity of seeds from different altitudes is relevant to determine the reproductive efficiency of a species (Vera 1997) [13].

Conclusion

Consequently, the best pre-sowing treatment for alleviating dormancy along with enhanced germination was gibberellic acid @ 2000 ppm at 850m A.S.L. This resulted in the best seed germination percentage in both altitudes. On the other hand, the lowest seed germination percentage was found under the traditional hot ash treatment and control condition.

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